

Effects of High-Frequency Electromagnetic Field on Ghrelin`S Quantitative Changes and Animal Memory

Khatuna Dondoladze, PhD Student

Ivane Javakhishvili Tbilisi State University, Tbilisi

Marine Nikolaishvili, Prof. and Head of Department

I.Beritashvili Center of Experimental Biomedicine,

Sokhumi State University, Tbilisi

David Zurabashvili, Prof.

Ivane Javakhishvili Tbilisi State University, Tbilisi

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Abstract

Ghrelin is one of the major ways of improving memory, which is approved by different amino acids ratio. However, reducing the concentration of Ghrelin helps to prevent the implementation of generated and stiffened nutritional act. The processes of stimulation cause various improvement of expressed behavior on positive stimulus. This is based on the type of overpass labyrinth for improving the reflexive behavior to two feeding test. These facts approve that the processes which was developed as a result of Ghrelin`s and EMF action and the central mechanisms of animals behavioral reaction on positive stimulus were connected to each other by morphofunctional and neurochemical organization.

Keywords: EMF, Ghrelin, Behavior

Introduction

An organism's habitat is unthinkable without stress. Healthy organism responds with non-specific reaction of the severe physical or psychological stimulus. Stress management physiological processes affect all systems of the body. When the stress is too strong or when the effect continues for a long time, the body is unable to cope with stress. This is the reason it damages human health. As a result of it, severe somatic or mental illness develops. Notwithstanding the cause (Neuropsychiatric, heat, cold, light, and other types of anthropogenic stress), the central nervous system has a direct role in the management of stress reaction. Consequently, a special place occupies the hypothalamic -pituitary - adrenergic axis in stress biology. Stress regulations cascading neurohormones is widely studied.

During stress, the whole organism starts to work in an extreme mode. The changes that occur in the alarm stage helps to prepare the body's adaptive mechanisms. These changes involves: better concentration and focus which makes life easier and more productive; brain instantly makes a decision; and fear becomes a depression.

It is known that in the fear inhibition, new environment adaptive mechanism is involved in the limbic system, in amygdala, hippocampus, and hypothalamus. The hippocampus is responsible for memory formation. On the other hand, the amygdala is involved in emotional behavior and the emotional charge of the background in the current generation of memory. Furthermore, the structure of the brain plays a critical role in fear management process; particularly, its significant role is the management of chronic stress (Carleen, 2013).

In recent years, studies have shown that stress increases the hormone Ghrelin's plasma concentrations that affect the severity of the feeling of fear and amygdala. Hormone Ghrelin was discovered by scientists about fifteen years ago. Ghrelin is a peptide hormone produced by ghrelinergic cells in the gastrointestinal tract which functions as a neuropeptide in the central nervous system and at the receptors located in the hypothalamus, hippocampus, and amygdala. At the same time, it acts on appetite regulation and it is involved in the development of diseases such as neurogenic anorexia (Monteleone, 2015). In addition, it affects learning and memory mechanism (Zhao, 2014) and improves knowledge and remembering processes (Spencer, 2015).

Ghrelin's concentration increased during chronic stress. Ghrelin improves learning and memory process. Subsequently, there is a hypothesis (Meyer, 2013) which states that during Stress, Ghrelin's concentration long gain contributes to the stability of post-traumatic stress disorder resistance (Schellekens, 2013).

Nowadays, the posttraumatic stress disorder neurobiological research axis is focused on monoamine neurotransmitters and HPA (both are stressful events mediated defense reactions). In addition, studies have shown changes in the hippocampus and amygdala, which is involved in the unconscious emotional stimuli processing. However, these facts show that hippocampal dysfunction prevents the formation of memory. The amygdala leads to the activation of noradrenergic vigilance and facilitates automatic coding and traumatic memories fixation.

It is also known that the electromagnetic field's (EMF) long-term impact on the body, such as the stresses-factor, causes emotional, perceptual, cognitive, memory, and learning processes pathology (Wilson, 1999).

It is approved and doubtless that electromagnetic field (EMF) has impact on the central nervous system, neuroendocrine cells, and hormones in the stomach too (Min, 2011). Emotional, perceptual, cognitive, memory, and

learning processes pathology in many cases was accompanied by long-term exposure to EMF's, thus changing the behavior.

Studies in rats showed decrease in learning, memory processes, and anorexia cases under a certain frequency of EMF (Mahdavi, 2014). On the other hand, Ghrelin's concentration must be increased in case of anorexia and is used in stimulating the learning processes.

If the electromagnetic field acts on organism like the factor of a stress, it should improve learning. This, however, can be explained by the influence of Ghrelin's increased concentration. However, the mechanism which include; why in the body of rats, under EMF, learning and memory processes were decreased. This was despite the fact that the increased concentration of Ghrelin was unknown.

Based on the above, we decided to study animal's reflexive behavior with two feedings and overpass type labyrinth test. Although, there is an influence of Ghrelin's quantitative change on rats memory under the high frequency electromagnetic field (EMF).

Material and Methods

Animals: Male Vistar rats, 5-6 weeks of age and weighing 200 g, were used in this study. The rat were housed for 1 week adaptation in a room on a 12-h light/dark cycle and was fed on a rat chow diet before the experiment. Rats were exposed under 600 Hz electromagnetic field (during 2, 3, 4, 5, 6 and 8 hours/day) for 6 days.

For reflexive behavior study, which was generated by positive stimulus (food), we used two meal feeding test. Experiments were conducted in 100X100X110X90 cm size trapezoidal box. We placed the meal near the longer wall, and the distance between them was 80 cm. The rat was placed on a starting camera (station), which was standing on the opposite wall. Therefore, we considered the proper response of 80-90% in the last three days to be a satisfied learning criteria.

Labyrinth test was conducted in overpass type labyrinth, which consisted 40-50 cm long strips attached to the 20 cm tall uprights. Also, we took single small bridges. By relocating them, we built some difficult labyrinth. Also, we were teaching the rats to run on the bridges and the stairs "arrival." At the end of the ladder, there was a box which consisted of animal's food. Tests interval was 2-3 minutes. Also, we considered earned strength criterion to be the run of the labyrinth 3 times without any mistake in the shortest possible way. Time spent on running the labyrinth was counted. It was counted beginning from the moment of setting the pitch, and it lasted until the rat returned to his den

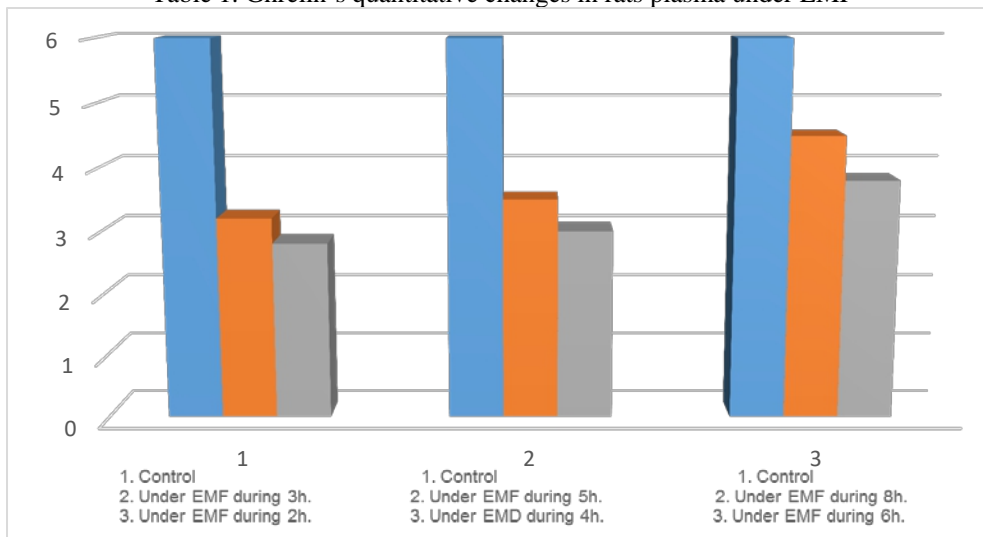
The Rat, Ghrelin ELISA, is used to measure and quantify Ghrelin (ISO 13485) levels.

Results

Hanger of rats for 24 hours, which caused increasing concentration of Ghrelin at the same time, was improving the output of differentiated nutritional act to the two feedings on the signal conditioning. On the other hand, without hunger, the behavior of rats obstructed the differentiated move to two feedings on the provided signal. Most of the animals of this kind (80%) didn't get out of the starting cage. When they got out (20%), they moved very slowly towards the feeding. However, their move to get the food was correct. If the animals who didn't get out were removed from the cage by force, then they went toward the feeding correctly. Additionally, the recovery of reaction to the conditioned signal with the two feeding was going gradually and was recovered fully on the third day. Thus, it turns out that reducing the concentration of Ghrelin prevents the implementation of generated and stiffened nutritional act. Based on some attempts, we studied high frequency EMF influence on Ghrelin's quantitative change. Also, we discovered that the Ghrelin's concentration increases in rat's plasma (Table 1).

When we studied rats 'conditioned reflexive behavior with two feeding test under EMF exposure during 6 hours, it was found that 9 Ghrelin's concentration increase as a result of EMF's action (Table 1). This caused conditioned and reinforced differentiated feeding reactions simplification, which was expressed by reduction in the time spent to come from the starting point to feeding box.

Table 1. Ghrelin's quantitative changes in rats plasma under EMF



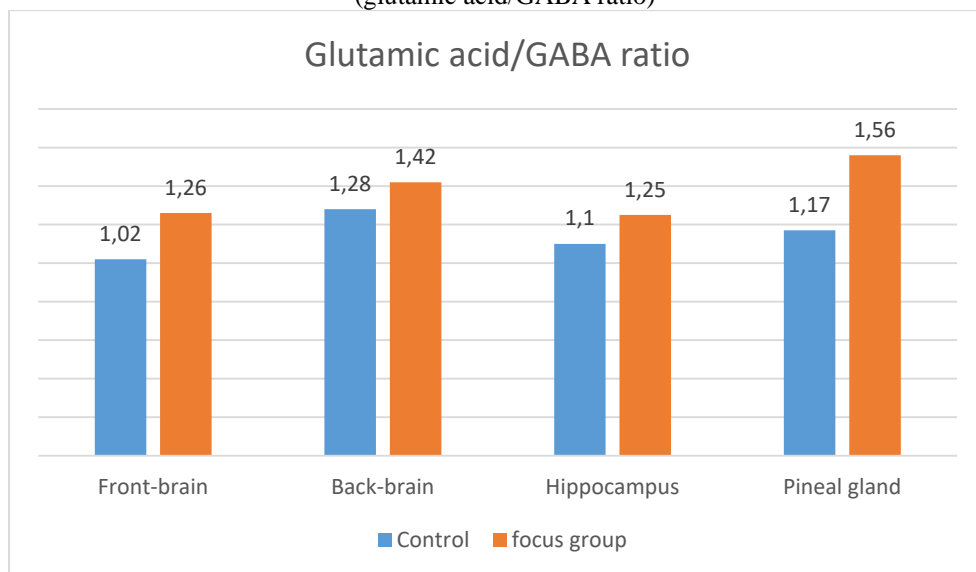
The following tests were directed towards the studies of amino acids which are participating in inhibition and excitation. As it is known, functional state of CNS depends on the ratio of inhibition and stimulation of

amino acids. An important aspect is also the ratio that exists between glutamate and glutamine (Nikolaisvili, 2016).

The foregoing may allow us to discuss the amount of ammonia in CNS. Increase of ammonia denotes excess process of excitation in CNS and, vice versa, a decrease in the amount of ammonia indicates activity of inhibition processes. Therefore, ammonia is considered as a chemical indicator of CNS functional state (Chichinadze, 2014). Following the said argument, we thought it necessary to calculate the ratios of glutamine acid in relation to GABA and glutamine acid.

The second table indicates that after 6 hours of exposure, high frequency EMF ratio of glutamine acid to glutamine, in the anterior part of brain hemisphere, increases for 64 %. In the other parts, the changes are not noticed. Thus, the ratio of glutamine increases to GABA in the hippocampus for 19% and in epiphysis for 24%. Changes that occur in free amino acid metabolism were expressed in animal's behavioral acts, particularly the behavior that is caused by conditioned behavior.

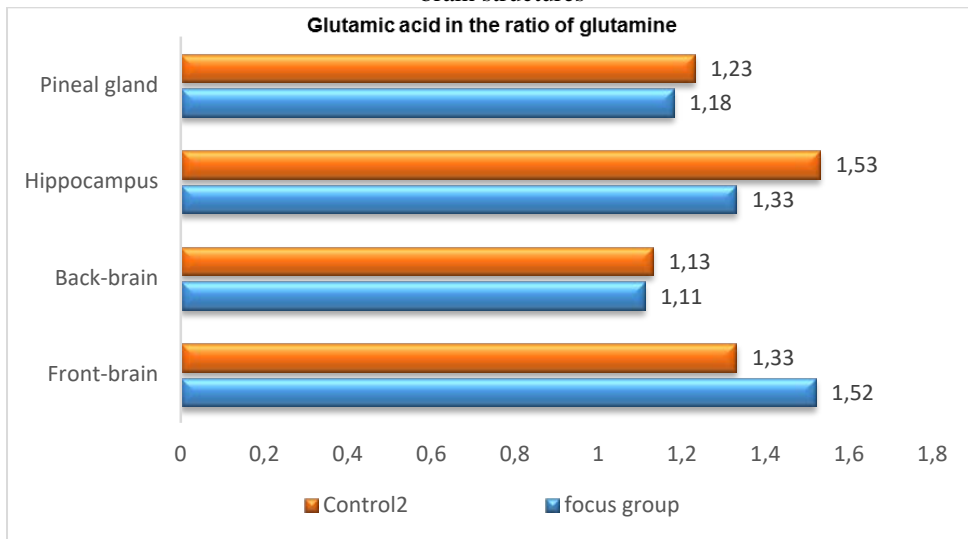
Figure 2. The influence of high-frequency EMF amino acid's distribution in brain structures (glutamic acid/GABA ratio)



Discussion

Thus, these results let us conclude that amino acids changes. Also, increasing Ghrelin's concentration which is caused by EMF explosion is expressed in animals behavioral acts. Particularly, amino acids which participate in stimulation are activating the positive stimulator of the behavioral act which is caused by feeding. However, the behavior has been generated for the problem to extend up to 3 days after it returned to the given level.

Figure 3. High-frequency EMF influence distribution of glutamic acid/glutamine ratio in the brain structures



In many experiments, we studied conditioned behavior caused by positive stimulus. In overpass labyrinth test, labyrinth consisted of 40-50 cm long bars, which were attached on the pickets for 20 cm long. Through this way, we took some little bridges and built complex labyrinth by relocating the bridges. Consequently, we taught rats how to run on these bridges and to move down on special stairs. Ladder ended by feeding box.

In the labyrinth, the way of developing conditional behavior was to place the rat, on the bridge nearby the ladder, until learning. It stopped for a while and when it saw the food, it started coming down the stairs carefully. Then we made the task more difficult. The rat was further placed on the bridge after stabilizing the time spent on coming down the stairs. Also, we placed the rat on the starting station and taught it how to run the whole labyrinth (starting station, running the bridges and labyrinth, and going back to the den). The animal ran the labyrinth 3 times a day. The interval between attempts was 3-4 minutes. Thus, we considered the task completed when the labyrinth was ran by a shortest way without any mistake. The time which was spent to run through the labyrinth was marked, and we started to count it from the moment the animals was placed on the starting station. However, it continued till the rat returned to the den. Also, the bridges in the labyrinth were numbered.

These bridges, however, enabled us to restore, after the experiment, the animal movement trajectory. Rats which were near the feeding were placed in the last bridge so as to quickly find the ladder and return to the box feeding. After 6 hours after exposure under EMF, we placed rats in the starting station. In achieving these goals, the rats moved much. There were

many random and chaotic movements on the bridges. Thus, they often treated deadlock labyrinth, and sometimes they returned back to the starting station. From this place, they started to move again. As soon as the rats were in the bridge, they went down on the ladder and went to the feeding box where they couldn't get the food. In the next experiment, rats started to move in the bridge more frequently; and at last, they choose the shortest way through which they got to the feeding. Some rats reached the goal faster than the control group rats.

After 2-3 attempts, most of the rats could decide the task on the first experiment day. Firstly, the time that was spent on running the labyrinth was enough which is as much as 50-60 seconds. Then after 2-3 days, it reduced to 10-15 seconds. This group reached the goal 2-3 times faster than the control group rats.

Thus, rats which were under EMF, which caused increase in the concentration of Ghrelin in plasma, and also increase in the ratio of amino acids in the irritation and retention were the obvious expression of activating the process of stimulation of this kind of memory which is caused by positive stimulation.

In the plasma of rats, increasing Ghrelin's concentration is possible by the action of EMF (Min Eui H. et al., 2011). However, mechanism which causes these changes aren't known yet. It is possible that Ghrelin plays an intermediary role in neuro-endocrinian behavioural and stress process (Asakawa, 2001). According to this, the stomach is not only an appetite regulation organ, but it is important in stress regulation processes.

Conclusion

By the results obtained above, we can conclude that Ghrelin is one of the major ways of improving this kind of memory, which is approved by different amino acids ratio. The processes of stimulation cause improvement of expressed behavior on positive stimulus on the type overpass labyrinth for improving the reflexive behaviour to two feeding test. These facts approve that the processes which developed as a result of Ghrelin's and EMF action and central mechanisms of animals behavioral reaction on positive stimulus are connected to each other by morphofunctional and neurochemical organization.

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